REMARKS

I. Introduction

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claims 31-35 are currently being amended, and claims 36-42 are being added.

This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

Support for the claim amendments and newly added claims is found throughout the specification. Exemplary support is found in the specification as indicated in the chart shown below.

Claim #	Exemplary Support in the Specification
31	Page 7, lines 30-32
32	Original claim 5
33	Page 7, lines 30-32
34	Page 7, lines 30-32
35	Page 7, lines 30-32
36	Figure 5; page 4, line 34, through page 5, line 5
37	Figure 5; page 4, line 34, through page 5, line 5
38	Figure 5; page 4, line 34, through page 5, line 5
39	Figure 5; page 4, line 34, through page 5, line 5
40	Figure 5; page 4, line 34, through page 5, line 5
41	Page 7, line 36, through page 8, line 2
42	Page 7, line 36, through page 8, line 2

Upon entry of this Amendment, claims 31-42 will remain pending in the application. Because the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

II. Response to Issues Raised by Examiner in Outstanding Office Action

A. Request for Information Under 37 C.F.R. § 1.105

The Examiner requires Applicants to provide all pertinent information regarding the source of the protein used as a standard. The Examiner notes that the specification at page 7 teaches the use of an hPTH (1-84) standard.

Applicants attach as Exhibit 1 a Declaration of Dr. Kaare Gautvik pursuant to 37 C.F.R. § 1.132, which relates to the hPTH (1-84) standard referred to at page 7 of the specification. The hPTH (1-84) standard is synthetic hPTH (1-84) obtained from chemical supply companies, including Peptide Institute Protein Research Foundation, Penninsula Laboratories, Sigma, and Bachem. The Declaration describes an SDS-PAGE gel in which 0.2 μg of hPTH (1-84) was loaded into various lanes. The hPTH (1-84) was obtained from: (a) Peptide Institute Protein Research Foundation, Penninsula Laboratories, Sigma, or Bachem, or (b) produced according to the claimed invention. A picture of the SDS-PAGE gel is provided in Exhibit B of the attached Declaration.[1] The SDS-PAGE gel confirms that the synthetic hPTH (1-84) obtained from Peptide Institute Protein Research Foundation, Penninsula Laboratories, Sigma and Bachem contains impurities as compared to the hPTH (1-84) produced according to the claimed invention.

The synthetic hPTH (1-84) obtained from Peptide Institute Protein Research Foundation, Penninsula Laboratories, Sigma and Bachem that was described as a PTH standard in the specification on page 7 contained impurities as shown in the SDS-PAGE gel provided as Exhibit B in the attached Declaration. However, the synthetic hPTH (1-84) obtained from Peptide Institute Protein Research Foundation, Penninsula Laboratories, Sigma and Bachem was a useful standard for confirming the identity of hPTH (1-84) produced according to the claimed invention.

[1] Exhibit B is a reproduction of a photograph of the SDS-PAGE gel.

B. Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claims 31-35 are rejected by the Examiner under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite. Applicants respectfully disagree with the Examiner and traverse this ground for rejection.

For the sole reason of expediting prosecution of the present application, Applicants have deleted the term "substantially homogenous" from the claims and have amended claims 31 and 32 to recite that "wherein said hPTH (1-84) is more than 90% (or 95%) pure." Support for this amendment is found in the specification on page 7, lines 30-32 and in original claim 5. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

C. Claim Rejections - 35 U.S.C. § 102

1. Rejection of Claims 31-35 Over Brewer

Claims 31-35 are rejected by the Examiner under 35 U.S.C. § 102(b) as being allegedly anticipated by Brewer et al. (U.S. Patent No. 3,886,132) ("Brewer"). Applicants respectfully request reconsideration and withdrawal of the rejection.

a. Claims 31-32

With respect to claims 31 and 32, the Board refers to page 5 of the Examiner's Answer where the Examiner reasoned that Brewer's "preparation was pure enough to sequence 34 amino acid residues starting at the amino terminus of the protein. Thus, the protein as purified by Brewer et al. appears to be consistent with the limitations in the instant claims with respect to being 'substantially homogenous' hPTH." In response, the Board states that it is unclear from the specification what degree of purity is intended by "substantially homogenous." Applicants do not agree. However, to expedite prosecution, Applicants have amended claim 31 by replacing the term "substantially homogenous" with the term "more than 90% pure."

Furthermore, Applicants respectfully disagree with the Examiner's assertion that while it cannot be determined whether Brewer's protein is 95% pure, it seems likely that Brewer's protein is 95% pure because of Brewer's ability to obtain a sequence for 34 residues. *See* Answer page 5. As discussed in the attached Declaration of Dr.

Kaare Gautvik pursuant to 37 C.F.R. § 1.132 (Exhibit 2), the isolated hPTH described by Brewer (U.S. Patent No. 3,886,132) in column 2, lines 3-11 is less than 90% pure. Additionally, Brewer's ability to obtain a sequence for the first 34 amino acid residues of his final hPTH preparation by Edmond degradation does not mean that Brewer's final hPTH preparation was more than 90% pure. To the contrary, Brewer's sequence contained 3 serious amino acid errors indicating that Brewer's final hPTH preparation is not only less than 90% pure, but also will have significantly different biological and immunological properties as compared to the natural peptide.

(i) Brewer's Purification and Edmond Degradation Method

Brewer isolated hPTH from dried, defatted parathyroid tissue as described in column 2, lines 3-13. Brewer indicates that the final hPTH preparation was analyzed by disc gel electrophoresis and monitored by radioimmunoassay. However, Brewer fails to provide a picture of the gel or any other documentation regarding the purity of the final hPTH preparation. As a result, a determination of the purity of the final hPTH preparation can be based only on an analysis of the results of Brewer's N-terminal sequencing of the first 1-34 amino acid residues from the intact hPTH of 1-84 amino acid residue protein/peptide using Edman degradations employing the Beckman Sequencer, Model 890B. The year that the 890B model was introduced is believed to be 1973.

Brewer's Edman degredation involved a Beckman sequencer in which phenylthiohydantoin amino acids were identified by regeneration to the constituent amino acid by hydrolysis with hydroiodic acid. Identification of the amino acid derivatives was carried out by gas liquid chromatography and mass spectrometry. *See* column 2, lines 23-39, of Brewer. Brewer degraded 350 nanomoles of the final hPTH preparation on the Beckman Sequencer using a single cleavage of heptofluorobutyric acid at each degradation. The results of the degradation of the first 34 residues is shown in Figure 1. *See* column 2, lines 55-59.

(ii) The amino acid sequence reported for the first 34 amino acids of Brewer's final hPTH preparation contains 3 mistakes

The sequence results of Brewer's Edman degradation contained 3 mistakes, namely at position 22 (gln instead of glu), in position 28 (lys instead of leu) and in position 30 (leu instead of asp). The probable conclusion as to why Brewer failed to obtain a correct sequence of the first 34 amino acids of hPTH, is that Brewer's final hPTH preparation was impure - containing a significant amount of other peptides/proteins which seriously disturbed and blurred the results of the Edman degradation analysis. This is because the presence of contaminating proteins in Brewer's final hPTH preparation, dramatically increased the chances to obtain a faulty sequence for hPTH.

(iii) The presence of more than one peptide/protein in the original mixture leads to greatly increased probability for incorrect identification of amino acid residues

A major problem relating to correct identification of each amino acid residue via the Edman degradation sequence method is the presence of more than one peptide/protein in the original mixture. A feature of the Edman degradation sequencing method is that the material that is being analyzed is consumed during the process, rendering stepwise identification of each amino acid more uncertain as sequencing moves away from the start N-terminal residue. Another feature of the method is the accumulation of blocked peptides during the process which obscure the obtained results. These two features, along with impurities in the starting material, cause the absolute and relative amounts of non-natural hPTH molecules to increase substantially as a function of degradation steps (cycles), thus reducing the accuracy and fidelity of the amino acid derivative chromatogram readings. *See* the graph on the left side of the of the Beckman User Manual, "Determining Sequencer Sensitivity" (1983) (Exhibit B), which shows reduction in yield of each amino acid (reflecting the true loss of substance) as a function of the number of cycles.

The graph on the left side of the Beckman User Manual shows sequencing of apomyoglobin on a more modern Beckman instrument (developed about 10 years later

than the Model 890B utilized by Brewer). It is noted that "PTH-amino acid" as used in the Beckman reference, is shorthand nomenclature used by those of skill in the art to designate an amino acid of the method employed, and does not refer to parathyroid hormone. The graph depicts the quantitative recovery (yield) of different amino acid residues (shown in capital letters) as a function of degradation steps (cycles). As the amount of available material that is being analyzed is reduced, the yield of a given amino acid is correspondingly reduced, rendering contaminating proteins and other impurities to exert an increasingly negative effect on the accuracy of the readings of the chromatograms. For example, after cycle 20 in the apomyoglobin case, the yield of recovery of the next amino acid residue is less than 50% of the initial yield of material (the initial yield of apomyoglobin was 63.8%). This technology represents at least a 10 year advancement in technology from the filing date of Brewer et al., suggesting a poorer yield for Brewer's material by cycle 20.

Typically, sequencing mistakes start to occur after the first 15-20 amino acids in the peptide are analyzed by Edmond degredations. In the initial cycles of Edmond degradations, when the amount of material that is being analyzed is large, the peaks in the chromatogram representing the major component of the material (hPTH) are larger than the peaks in the chromatogram representing the other components in the material (contaminants). (*See* the chromatograms on the right side of the page of the Beckman User Manual, "Determining Sequencer Sensitivity" (1983)) (Exhibit B). See especially the reduction in leu peak height given as recovered picomoles (yield), which falls dramatically after cycle 10. Note also that at line 2, page 2 of the Beckman User Manual, it strongly recommends that "[b]ackground subtraction or 'data enhancement' (if any) should be indicated" (line 2 page 2). "Background" refers to chromatographic noise due to impurities of all kind (see above). It was not an acceptable standard in 1975, nor is it acceptable today, to fail to include copies of genuine chromatograms of amino acid derivative separation for sequences determined by Edmond degradation. However, Brewer did not include copies of his chromatograms in the cited reference.

In the later cycles of Edmond degradation, when the amount of correct available/remaining material that is being analyzed is much smaller, the peaks in the chromatogram representing the major component (hPTH) are comparable in size to the

peaks in the chromatogram representing the other components in the material (contaminants). Therefore, the scientist is forced to "guess" which peak represents the major amino acid derivative in the hPTH sequence and which peak represents the other components (contaminants). The fact that the hPTH sequence disclosed by Brewer in Figure 1 contains 3 mistakes (at residues 22, 28 and 30) suggests that Brewer's final hPTH preparation contained contaminants and is clearly less than 90% pure.

(iv) Gas liquid chromatography causes separation difficulties with asparaginyl, glutaminyl, and lysyl derivatives

As discussed above, after performing Edmond degradation, Brewer identified amino acid derivatives by gas liquid chromatography and mass spectrometry. However, as described on page 281 of *Protein Sequence Determination* (2nd revised and enlarged edition) (1975), Edited by Saul B. Needleman (Springer-Verlag, Berlin-Heidelberg-New York) (Exhibit C), the use of gas liquid chromatography is known to cause separation difficulties with several amino acid residues including asparaginyl, glutaminyl, lysyl and other amino acid derivatives.

The mistakes in Brewer's sequence involve asparaginyl, glutaminyl, and lysyl derivatives. To at least partly overcome these separation difficulties, Brewer could have employed other liquid phases and modifying reagents. However, Brewer does not indicate that such agents were used.

(v) Brewer started with a much less than recommended amount of hPTH for sequencing.

Brewer assumes that 350 nanomoles of his final hPTH preparation were degraded on the Beckman Sequencer. As noted on page 34, line 14, of the specification, the molecular weight of hPTH is 9000 Daltons. Therefore, if Brewer's final hPTH preparation was truly "pure" hPTH, then this would mean that Brewer isolated at least 3.15 mg of hPTH (as described below, it is highly likely that Brewer's final hPTH preparation contained much less than 3.15 mg of intact, pure hPTH). 3.15 mg of protein, however, is insufficient for obtaining accurate sequence results, as clearly stated in Hermodson et al, *Biochemistry*, *11*:4493-4501 (1972) (*see* page

4497, second column, next last paragraph) (Exhibit D), which suggests a recommended sample size for the initial sequencing cycle of 7-10 mg.

An insufficient amount of starting material makes sequencing accuracy and fidelity in interpreting chromatograms much more susceptible to serious mistakes. Brewer's amount of starting material was insufficient, and as a result, the amino acid sequence Brewer reported for the first 34 amino acids of Brewer's final hPTH preparation contained 3 mistakes.

(vi) It is unlikely that Brewer could have isolated 3.15 mg of hPTH from parathyroid gland adenomas that was more than 90% pure

As noted above, 350 nanomoles of hPTH would be about 3.15 mg of hPTH. Brewer fails to specify the initial weight of the parathyroid tissue from which Brewer isolated the final hPTH preparation. However, if 1% of the total weight of the parathyroid tissue comprised hPTH (this is most likely an over estimation), then in order for Brewer to have isolated 3.15 mg of hPTH, he would have had to start out with 315 mg of parathyroid tissue. Since adenomateous hPTH gland weighs approximately 10-20 mg, this would mean that Brewer isolated hPTH from probably more than 16 glands. It would be impossible for a skilled artisan to purify 3.15 mg of hPTH from 16 glands. Therefore, a more likely explanation is that Brewer's final hPTH preparation contained a significant amount of contaminants and was less than 90% pure.

b. Claims 33-35

With respect to claims 33-35, the Examiner asserts that the product-by-process limitations are drawn to recombinant production of the claimed "substantially homogenous hPTH (1-84)", which as been found anticipated by Brewer, as affirmed by the BPAI. Applicants have amended claims 31 and 32 by deleting the term "substantially homogenous" from the claims and amending claims 31 and 32 to recite that "wherein said hPTH (1-84) is more than 90% (or 95%) pure." As discussed above, the PTH of Brewer is less than 90% pure. Therefore, the product of claims 31 and 32 is not anticipated by Brewer and claims 33-35 are therefore not anticipated by Brewer as well.

2. Rejection of Claims 31-35 as being Allegedly Anticipated by Applicant's "standard" hPTH (1-84)

The Examiner asserts that the specification at page 7 teaches the use of an hPTH (1-84) standard to compare and assess the results of the purification process. The Examiner asserts that for the "standard" to have been useful for such comparison, it itself must have met the limitations of the pending claims.

Applicants respectfully disagree with the Examiner. As discussed above, attached as Exhibit 1 is a Declaration of Dr. Kaare Gautvik pursuant to 37 C.F.R. § 1.132, which relates to the hPTH (1-84) standard referred to at page 7 of the specification. The hPTH (1-84) standard is synthetic hPTH (1-84) obtained from chemical supply companies including Peptide Institute Protein Research Foundation, Penninsula Laboratories, Sigma, and Bachem. The Declaration describes an SDS-PAGE gel in which 0.2 μg of hPTH (1-84) obtained from the chemical supply companies, or produced according to the claimed invention, was loaded into various lanes. A picture of the SDS-PAGE gel is provided as Exhibit B in the attached Declaration. The SDS-PAGE gel confirms that the synthetic hPTH (1-84) obtained from chemical supply companies contains impurities as compared to the hPTH (1-84) produced according to the claimed invention.

The synthetic hPTH (1-84) obtained from chemical supply companies and that was described as a PTH standard in the specification on page 7 contained impurities as shown in the SDS-PAGE gel provided as Exhibit B in the attached Declaration. In contrast, the hPTH of the claimed invention is more than 95% pure. Therefore, the synthetic hPTH that was used as an hPTH standard does not anticipate the purified hPTH of the claimed invention. Additionally, even though the synthetic hPTH that was used as an hPTH standard contained impurities, it was still a useful standard for confirming the certain identity characteristics of hPTH (1-84) produced according to the claimed invention.

3. New Claims 36-42 are not Anticipated by Brewer

New claims 36-42 are not anticipated by Brewer because these claims are directed to hPTH (1-84) comprising a sequence containing at least one amino acid selected from the group consisting of a glutamate at position 22, a leucine at position 28, and an aspartate at position 30.

As shown in Figure 1 of Brewer, the amino acids at positions 22, 28, and 30 are glutamine, lysine and leucine, respectively. In contrast, the amino acids at positions 22, 28, and 30 in the hPTH (1-84) of claims 36-42 are glutamate, leucine, and/or aspartate, respectively (see sequence alignment provided below). Therefore, claims 36-42 are not anticipated by Brewer.

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Brewer:     1  SVSEIQLMHNLGKHLNSMERVQWLRKKKQLVHNF 34
               SVSEIQLMHNLGKHLNSMERV+WLRKK Q VHNF
Invention:  1  SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF 34
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D. Claim Rejections - 35 U.S.C. § 103

1. Rejection of Claims 31-35 Over Breyel or Mayer et al., in view of Kaisha et al. and Brewer et al.

Claims 31-35 are rejected by the Examiner under 35 U.S.C. § 103 as being allegedly obvious over Breyel et al. ("Synthesis of mature human parathyroid hormone in Escherichia coli," *Third European Congress on Biotechnology*, Vol. 3, p. 363-369 (1984)) ("Breyel") or Mayer et al. (EP 0 139 076) in view of Kaisha et al. (GB 2 092 596) ("Kaisha") and Brewer et al. (U.S. Patent No. 3,886,132) ("Brewer")

The Examiner asserts that although Breyel fails to disclose a hPTH that was purified from bacterial cell extracts, and Mayer fails to teach purification to the degree recited in the rejected claims, a person of ordinary skill in the art would have been motivated to purify the hPTH as taught by Breyel or Mayer using the protocol suggested by Brewer because Kaisha teaches the desirability of making large quantities of hPTH. Applicants respectfully request reconsideration and withdrawal of the rejection.

a. The Rejection is Flawed Because the Purification Method of Breyel or Mayer Could not be Used to Purify Brewer's Biological Material

A proper rejection for obviousness under § 103 requires consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition, or device, or carry out the claimed process and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 493, 20 USPQ2d 1438 (Fed. Cir. 1991).

The Examiner has failed to establish a *prima facie* case of obviousness. A person of ordinary skill in the art would know that the purification procedure used to purify the hPTH of Breyel or Mayer could not be the same purification procedure used to purify the hPTH of Brewer. This is because the hPTH of Breyel and Mayer were derived from entirely different sources than the hPTH of Brewer.

Specifically, the hPTH of Brewer was purified from dried, defatted parathyroid tissue. In contrast, Breyel and Mayer disclosed expression of hPTH in *E. coli* (*see* page 363, "Summary," of Breyel and page 12, line 7 of Mayer). *E. coli* has endogenous exopeptidase and endopeptidase activity which cleaves internal protease sensitive domains in PTH. *See* Mathavan et al., "High Level Production of Human Parathyroid Hormone in *Bombyx mori* Larvae and BmN Cells Using Recombinant Baculovirus," *Gene, 167:*33-39, at 34 (1995) (Exhibit 3). Therefore, the hPTH of Breyel and Mayer contained *fragments* of hPTH. *See e.g.,* page 2, line 34, through page 3, line 8, of the present specification, where it is noted that Breyel demonstrated *E. coli* degradation of human PTH.

The cited art would not have suggested to those of ordinary skill in the art that they should, or could, purify the hPTH of Breyel or Mayer utilizing the purification procedure disclosed in column 2, lines 3-13, of Brewer because a person of ordinary skill in the art would know that the hPTH of Breyel and Mayer contained significant amounts of hPTH fragments, since the hPTH material was expressed in *E. coli*.

Whereas the hPTH of Brewer did not contain hPTH fragments, since it was obtained from dried, defatted parathyroid tissue.

b. One of Skill in the Art Would not Have a Reasonable Expectation of Success in Applying the Purification Process of Brewer to the hPTH Fragments of Breyel or Mayer

Furthermore, a person of ordinary skill in the art would not have had a reasonable expectation of success in applying the hPTH purification procedure of Brewer to the hPTH fragments of Breyel and Mayer to obtaining hPTH(1-84) that meets the present claim limitations. A person of ordinary skill in the art would know that purifying hPTH(1-84) from hPTH fragments would be extremely difficult because the chromatographic properties of hPTH fragments would be similar to the chromatographic properties of intact hPTH(1-84). Therefore, a person of ordinary skill in the art would not expect the generic three step purification procedure described in col. 2, lines 3-13, of Brewer to purify the hPTH of Breyel and Mayer to yield intact hPTH (1-84) that is more than 90% pure.

At best, the Examiner is using an improper "obvious to try" standard, arguing that it would have been obvious to a person of ordinary skill in the art to purify the hPTH of Breyel or Mayer utilizing the purification procedure disclosed in col. 2, lines 3-13, of Brewer. However, "'obvious to try' has long been held to not constitute obviousness." *In re Deuel*, 34 USPQ2d 1210 (Fed. Cir. 1995).

Applicants also note that the hPTH of Breyel is not "mature" PTH, as "mature" PTH consists only of the known and correct 84 amino acids of PTH, and does not additionally include other fused amino acid residues. *See e.g.,* Mahoney (WO 84/01173) at page 12, lines 1-2 and 33-37; and page 13, lines 27-32, referring to a "mature" protein (Exhibit 3). Nor does "mature" PTH include chemical modifications well known to occur to most peptides synthesized in *E. coli*. *See, e.g.,* Høgset et al. ("Expression of Human Parathyroid Hormone in *E. coli*" *BBRC*, Vol. 166, p. 50-60 (1990)) (Exhibit 4); Kareem, B. et al. ("Translocation and Processing of Various Human Parathyroid Hormone Peptides in *E. coli* and Differentially Effected by Protein-A-Signal Sequence Mutations" *European Journal of Biochemistry*, Vol. 22, p. 893-900 (1994)) (Exhibit 5); and Kareem, B. et al. (A Method for the Evaluation of the Efficiency of

Signal Sequences for Secretion and Correct N-terminal Processing of Human Parathyroid Hormone Produced in *E. coli*" *Analytical Biochemistry*, Vol. 204, p. 26-33 (1992)) (Exhibit 6).

c. The Teachings of the Cited Art Fail to Disclose Each and Every Limitation of the Claimed Invention.

As discussed above, the PTH of Brewer et al. is less than 90% pure. However, amended claims 31 and 32 recite "wherein said hPTH (1-84) is more than 90% (or 95%) pure." None of Breyel et al., Mayer et al. or Kaisha et al. cure the deficiencies of Brewer et al. Therefore, claims 31-35 are not obvious over the combined teachings of Breyel et al., Mayer et al., Kaisha et al. and Brewer et al.

2. New Claims 36-42 are not obvious over Breyel or Mayer, in view of Kaisha and Brewer

New claims 36-42 are not obvious over Breyel or Mayer, in view of Kaisha and Brewer for the reasons discussed above.

III. CONCLUSION

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant(s) hereby petition(s) for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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